

# Физические методы исследования состава и структуры веществ

## Оптическая микроскопия: Лекция 3

### Геометрическая оптика



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# Learning Goals of this Course

Recent advancements in Earth and Planetary and Materials sciences make it possible, and even more desirable, to image geological and functional materials at the micro ( $10^{-6}$  m) and nanoscale ( $10^{-9}$  m) level. The complete characterization of functional, geological and planetary materials (Moon, Mars, asteroids, comets, Earth's interior), at micro and nano-scale can be achieved only when *full set of advanced techniques is used*. It includes (a) x-ray diffraction, (a) electron microscopic techniques (including EDX, electron diffraction, etc.) combined with (c) Raman scattering, and (d) atomic force microscopy. Each of these techniques provides a unique set of information and insight into the structure and properties of the geological and functional materials.

1. This course is designed to give the student a *fundamental background in advanced techniques* used in Geophysics, Planetary and Material sciences. The student will learn the principles and operation of each of the instruments.
2. At the end of this course you will understand how to get *interpretation of the results* (images or spectra) obtained by the advanced techniques.

# Learning Goals of this Course

At the end of this course you will:

- Understand how to determine atomic structure, chemical composition, chemical bondings and the elastic properties of minerals and functional materials
- Understand the basics of optical microscopy
- Have a good background in 2-D and 3-D image analysis
- Understand the operation and function of a electron microscopy
- Understand the operation and function of a x-ray diffraction technique
- Understand the operation and function of a Raman spectroscopy
- Learn about measurements of the elastic properties of solids
- Understand how to characterize minerals and functional materials under extreme conditions (high pressure and temperature)

# Human Sensing Systems

How living organisms, including human being, can get information about the world around us?

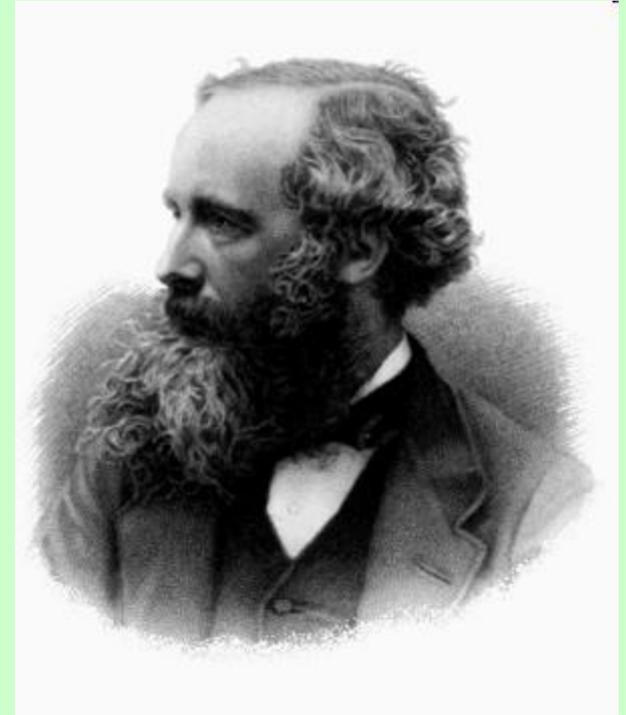
1. Using **Visual System (Light)**. Development: **Optical Microscopy**
2. Using **Auditory System (Sound)**. Development: **Acoustic microscopy.**
3. A system for **Sensing Touching (Tension)**. Development: **Atomic Force Microscopy.**
4. **Smelling System (Molecules):**

# Light is Electromagnetic Radiation

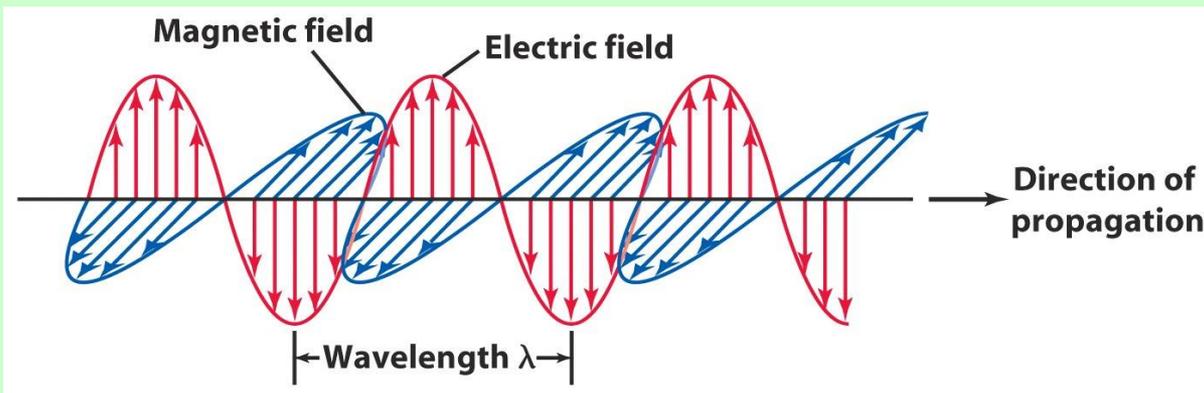
## The nature of light is electromagnetic radiation

In the 1860s, James Clerk **Maxwell** succeeded in describing all the basic properties of electricity and magnetism in four equations: the Maxwell equations of **electromagnetism**.

Maxwell showed that electric and magnetic field should travel in space with a constant speed called light velocity.

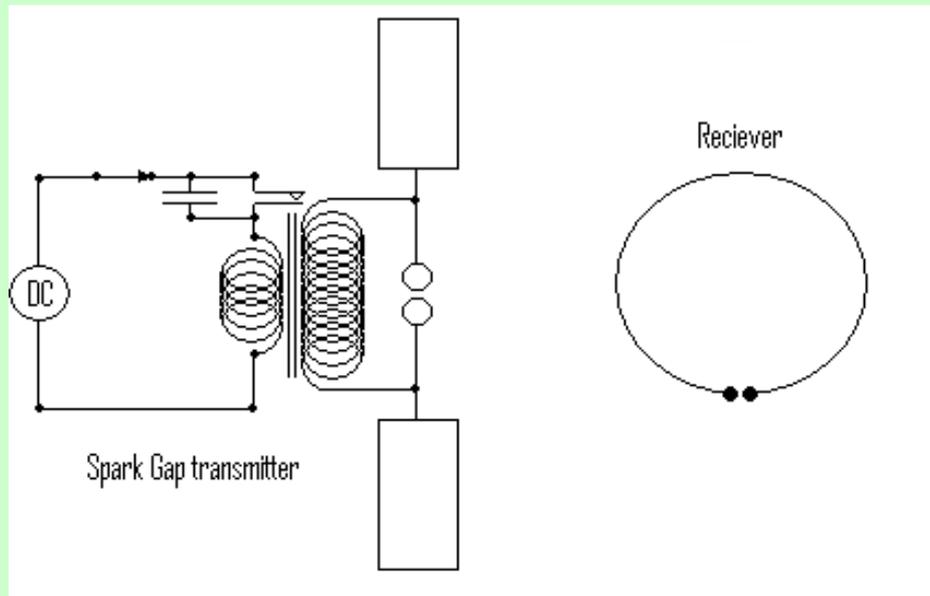


**James Clerk Maxwell**  
(1831 – 1879)



# Light is Electromagnetic Radiation

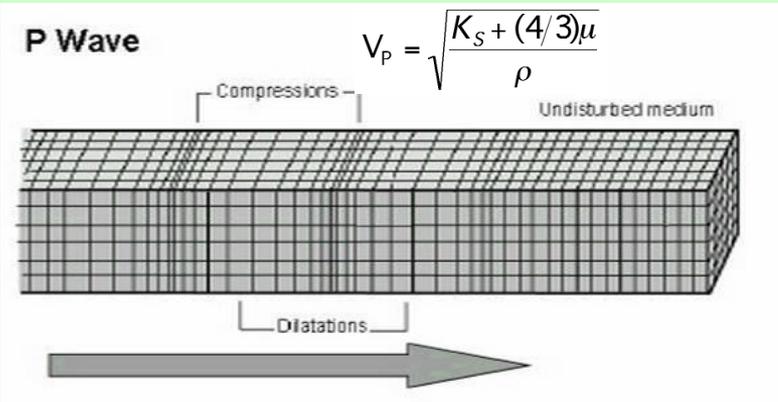
**Heinrich** was a German physicist who clarified and expanded the electromagnetic theory of light that had been put forth by Maxwell. He was the first to satisfactorily demonstrate the existence of electromagnetic waves by building an apparatus to produce and detect UHF waves.



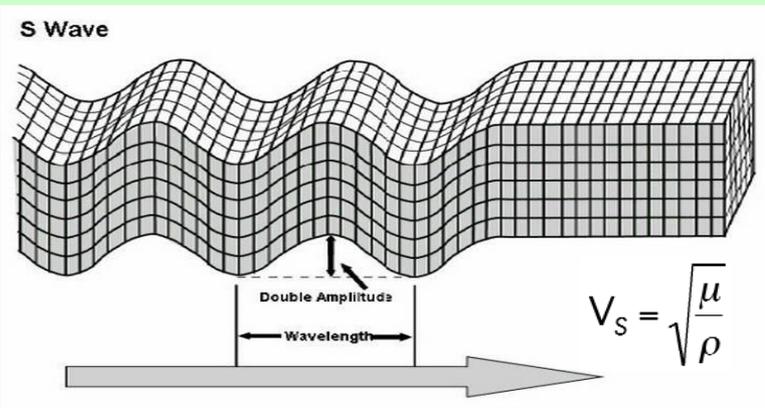
**Heinrich Rudolf Hertz**  
(1857 – 1894)

1887 experimental setup of Hertz's apparatus

# Sound Waves



Longitudinal (Compression) Waves - The particles of the medium undergo displacements in a direction parallel to the direction of wave motion.



Transverse Waves - The particles of the medium undergo displacements in a direction perpendicular to the wave velocity.

Galileo Galilei(1564–1642) but also Marin Mersenne (1588–1648), independently, discovered the complete laws of vibrating strings (completing what Pythagoras had started 2000 years earlier). Galileo wrote "Waves are produced by the vibrations of a sonorous body, which spread through the air, bringing to the tympanum of the ear a stimulus which the mind interprets as sound", a remarkable statement that points to the beginnings of physiological and psychological acoustics. Experimental measurements of the speed of sound in air were carried out successfully between 1630 and 1680 by a number of investigators, prominently Mersenne. Meanwhile Newton (1642–1727) derived the relationship for wave velocity in solids, a cornerstone of physical acoustics.

Sound is a travelling wave that is an oscillation of pressure transmitted through a solid, liquid, or gas, composed of frequencies within the range of hearing and of a level sufficiently strong to be heard, or the sensation stimulated in organs of hearing by such vibrations.

# Acoustical and Optical Microscopy

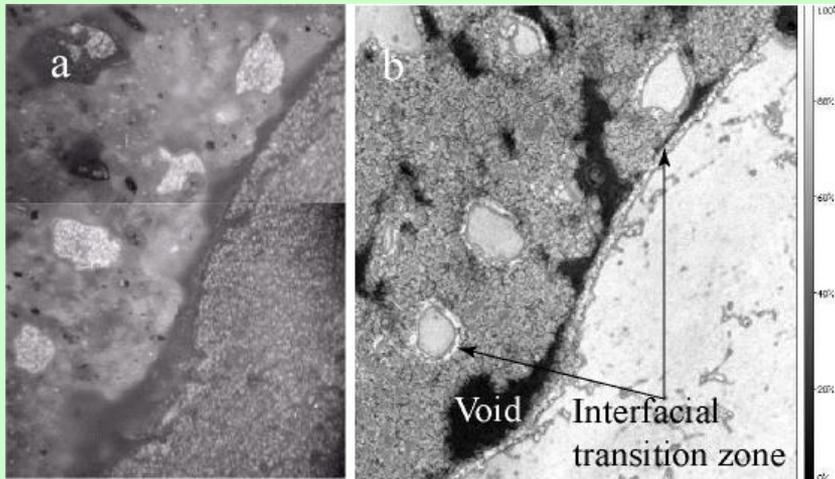


Optical Microscope

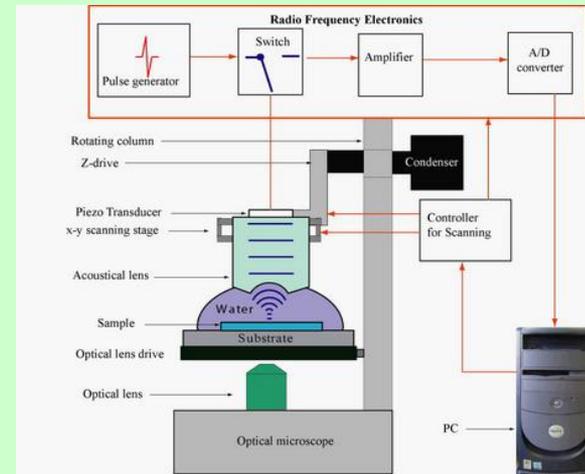
A **microscope** (from the Greek: μικρός, *mikrós*, "small" and σκοπεῖν, *skopeîn*, "to look" or "see") is an instrument to see objects too small for the naked eye.



Scanning Acoustical Microscope



Optical (a) and acoustical (b) images of concrete



# Light: Wavelength and Frequency

Frequency and wavelength of electromagnetic waves

$$f = \frac{c}{\lambda}$$

$f$  = frequency of an electromagnetic waves (in Hz)

$c$  = speed of light =  $3 \times 10^8$  m/s

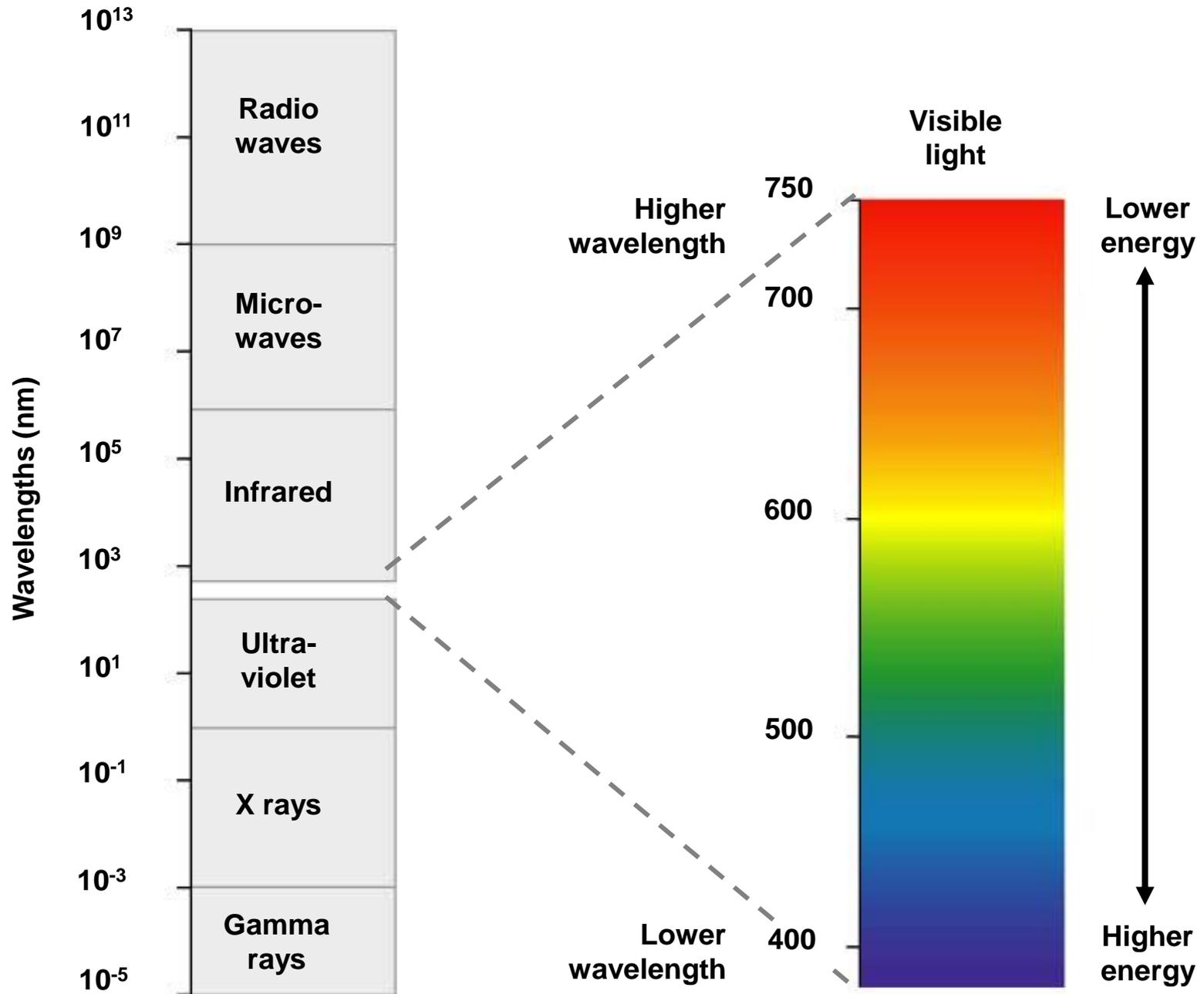
$\lambda$  = wavelength of the wave (in meters)

- Examples

- FM radio, e.g.,  $f = 96.3 \times 10^6$  Hz (Hawaiian station)  $\Rightarrow \lambda = 3.1$  m

- Visible light, e.g., red 700 nm  $\Rightarrow f = 4.3 \times 10^{14}$  Hz

# The Visible Light Spectrum



# Light Interaction with objects

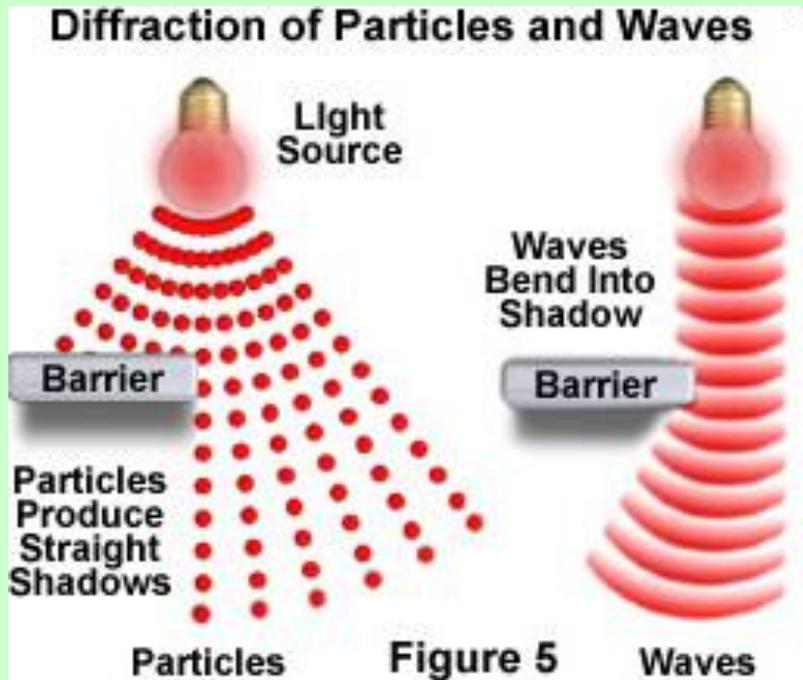
When a light wave strikes an object, a number of things could happen.

- Absorption: The light wave could be absorbed by the object, in which case its energy is converted to heat.
- Reflection: The light wave could be reflected by the object.
- Refraction. Refraction is the change in direction of a wave due to a change in its speed: direction change of a ray of light passing from one transparent medium to another with different optical density.
- Dispersion: *dispersion* is the phenomenon in which the phase velocity of a wave *depends* on its frequency: separation of light into its constituent wavelengths when entering a transparent medium.
- Diffraction: it is described as the apparent bending of waves around small obstacles and the spreading out of waves past small openings: light rays bend around edges - new wavefronts are generated at sharp edges

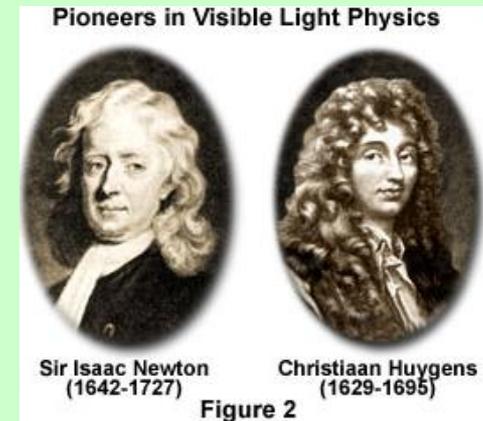
# Light Behaves as a Ray



# Light Behaved as a Wave



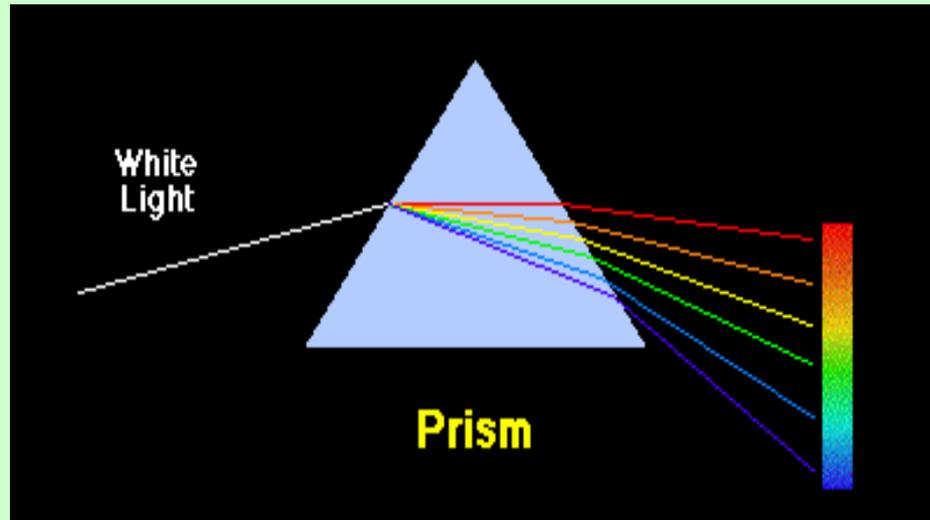
If the particles encounter the edge of a barrier, then they will cast a shadow because the particles not blocked by the barrier continue on in a straight line and cannot spread out behind the edge. On a macroscopic scale, this observation is almost correct, but it does not agree with the results obtained from light diffraction experiments on a much smaller scale.



Particles and waves should also behave differently when they encounter the edge of an object and form a shadow (Figure 5). Newton was quick to point out in his 1704 book *Opticks*, that "Light is never known to follow crooked passages nor to bend into the shadow". This concept is consistent with the particle theory, which proposes that light particles must always travel in straight lines.

# Dispersion

In optics, *dispersion* is the phenomenon in which the phase velocity of a wave *depends* on its frequency.



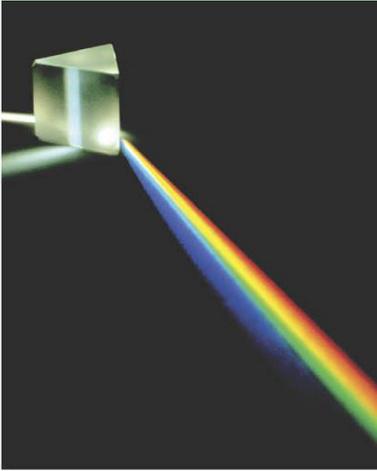
- Electromagnetic waves interact with the charged particles in matter and travel more slowly in transparent media than in a vacuum.
- The change in speed of the light wave causes the wave to refract.
- Since the velocity of an electromagnetic wave in a medium changes with wavelength, the amount of refraction depends on the wavelength.
- This effect is called *dispersion*.

# Dispersion

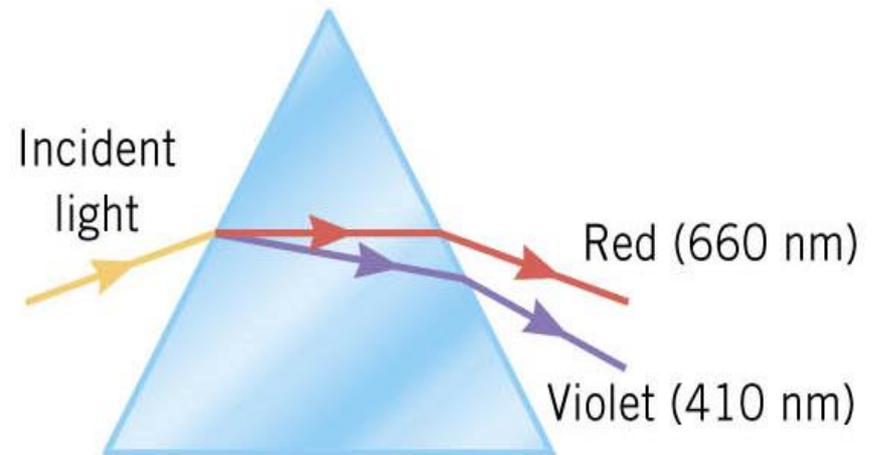
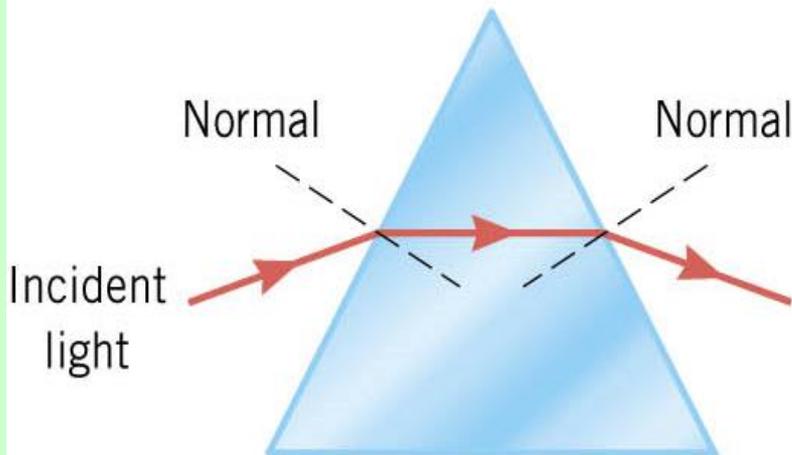
Index of refraction ( $n$ ) depends slightly on wavelength ( $\lambda$ )

Red light: it has the longest  $\lambda$  and the lowest  $n$

Red light: it has the shortest  $\lambda$  and the highest  $n$ ;  
it bends the most.



Glass prism



# Reflection and Refraction

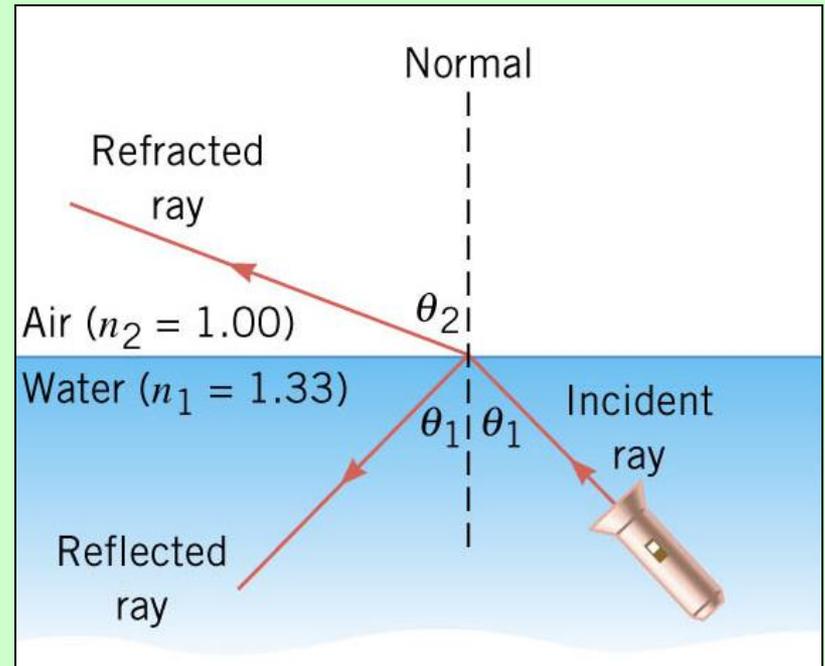
When a light ray travels from one medium to another, part of the incident light is *reflected* and part of the light is *transmitted* at the boundary between the two media. The transmitted part is said to be *refracted* in the second medium.

The angle of refraction depends on the indices of refraction, and is given by Snell's law:

$$n_1 \sin \theta_1 = n_2 \sin \theta_2$$

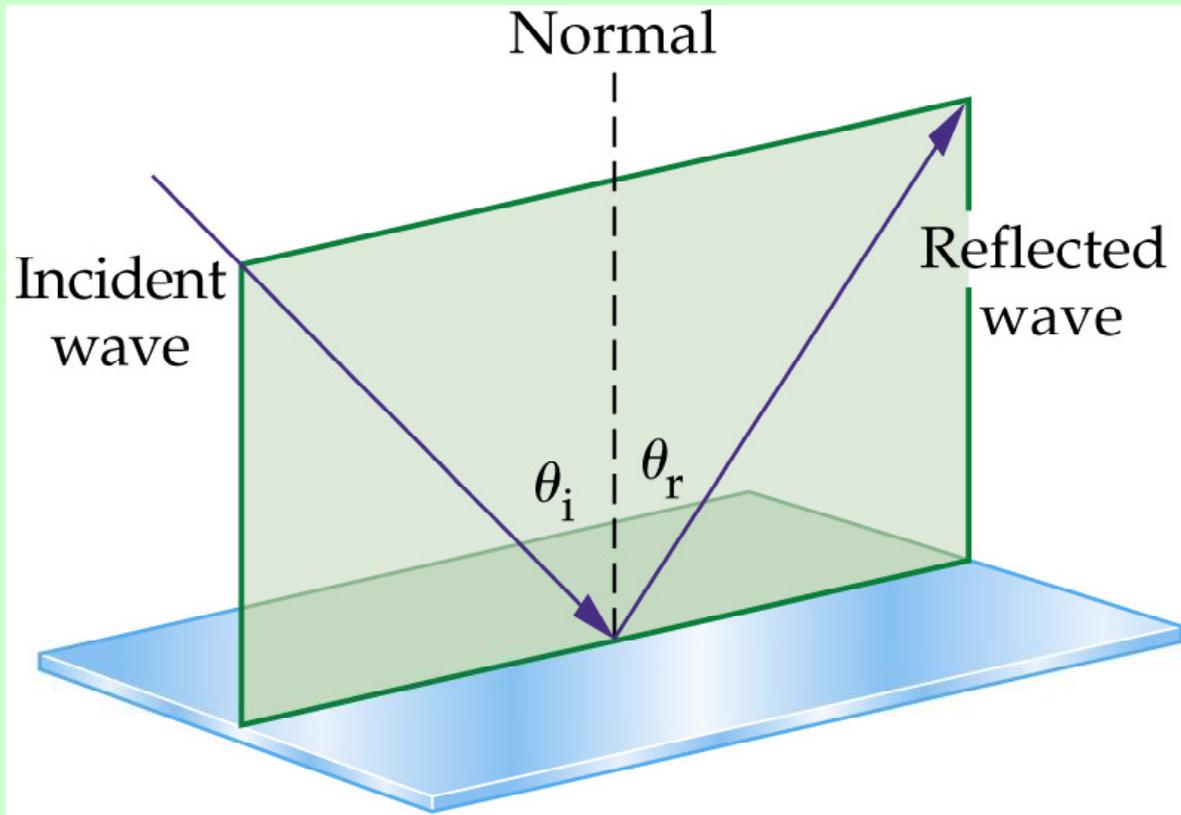
**Definition:** The ratio of the speed of light in vacuum,  $c$ , to the speed  $v$  of light in a given material is called the index of refraction,  $n$ , of that material

$$n = \frac{c}{v}$$



# The Law of Reflection

For specular reflection the *incident* angle  $\theta_i$  equals the *reflected* angle  $\theta_r$ :

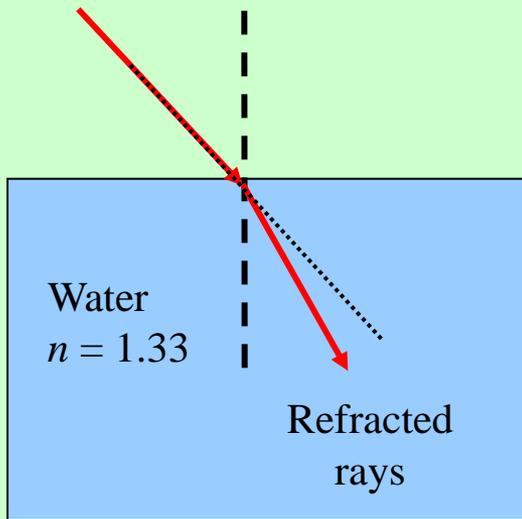


$$\theta_i = \theta_r$$

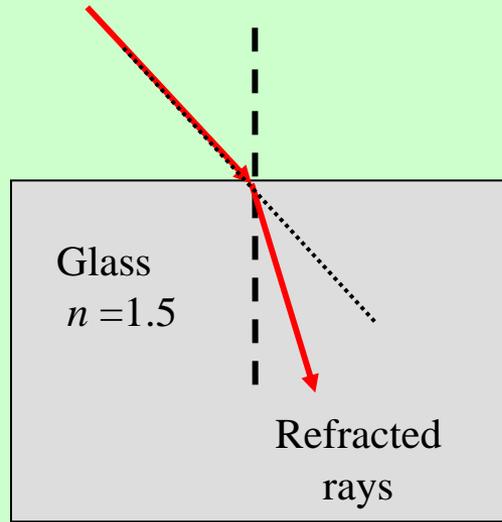
The angles are measured relative to the normal, shown here as a dotted line.

# Index of Refraction of Different Media

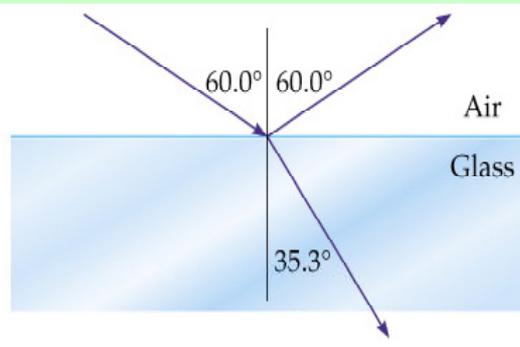
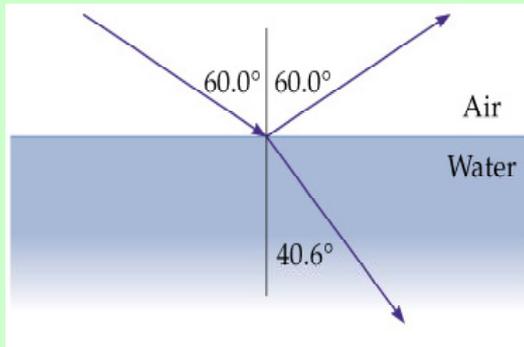
Incident ray



Incident ray



The refracted ray is bent more in the glass



**Table. Indices of Refraction**

Medium	$n=c/v$
Vacuum	1.0000
Air	1.0003
Water	1.33
Ethyl alcohol	1.36
Glass	
Fused quartz	1.46
Grown glass	1.52
Light flint	1.58
Lucite or Plexiglas	1.52
Sodium chloride	1.53
Diamond	2.42
$\lambda = 589 \text{ nm}$	

# Thin Lenses

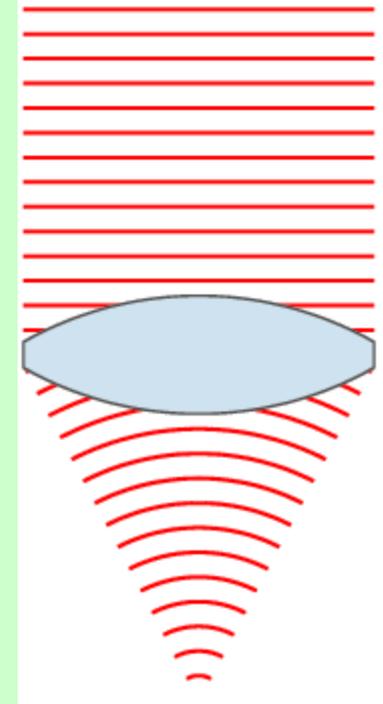


Not proper focusing



Proper focusing

# Thin Lenses



The oldest lens artifact is the Nimrud lens, which is over three thousand years old, dating back to ancient Assyria. David Brewster proposed that it may have been used as a magnifying glass, or as a burning glass to start fires by concentrating sunlight. Assyrian craftsmen made intricate engravings, and could have used such a lens in their work. Another early reference to magnification dates back to ancient Egyptian hieroglyphs in the 8th century BC, which depict "simple glass meniscal lenses" (Wikipedia, 2010)

# Thin Lenses; Ray Tracing

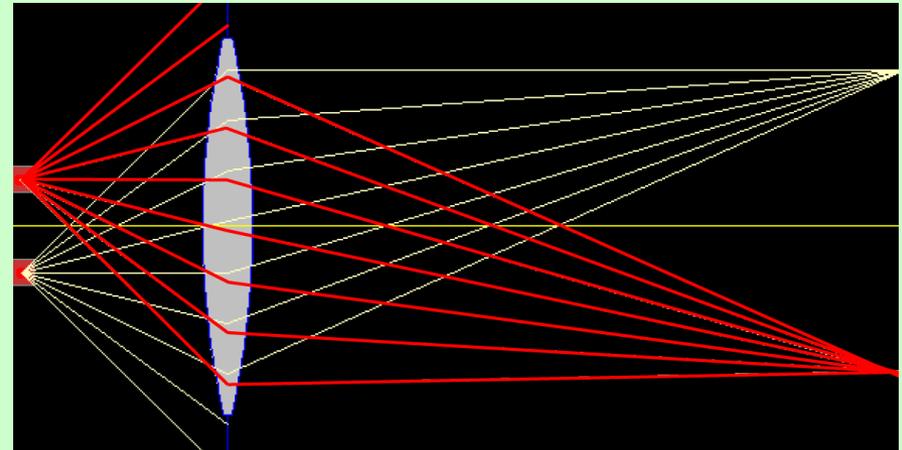
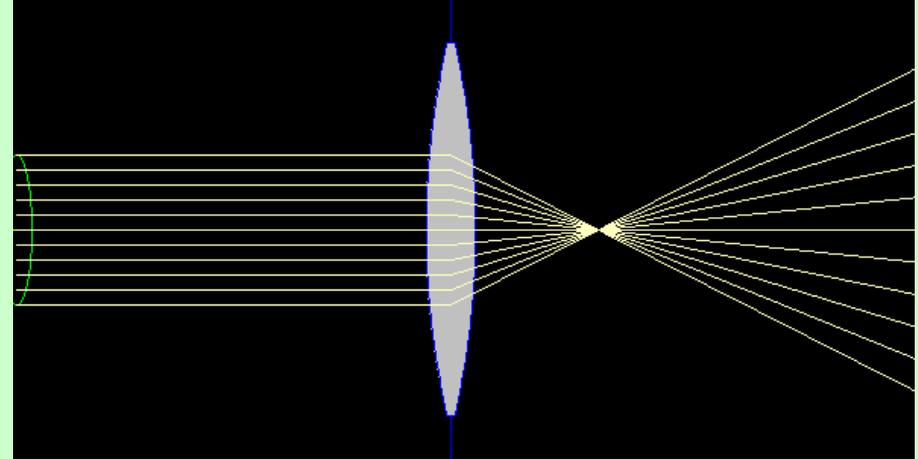
**Lens** is any element that focuses light to form images (“The Encyclopedia of Physics”, VNR,1985).

The *focal point is* the point at which rays of light, that were initially parallel to the to the symmetry axis of the lens or mirror, meet after passing through a convex lens, or reflecting from a concave mirror.

The power of a lens is the inverse of its focal length.

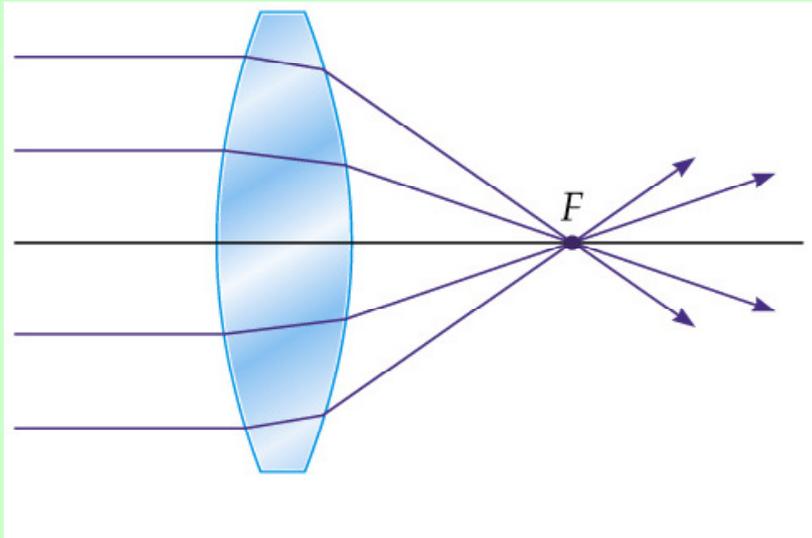
$$P = \frac{1}{f}$$

Lens power is measured in diopters,  $D$ .  $1 D = 1 \text{ m}^{-1}$

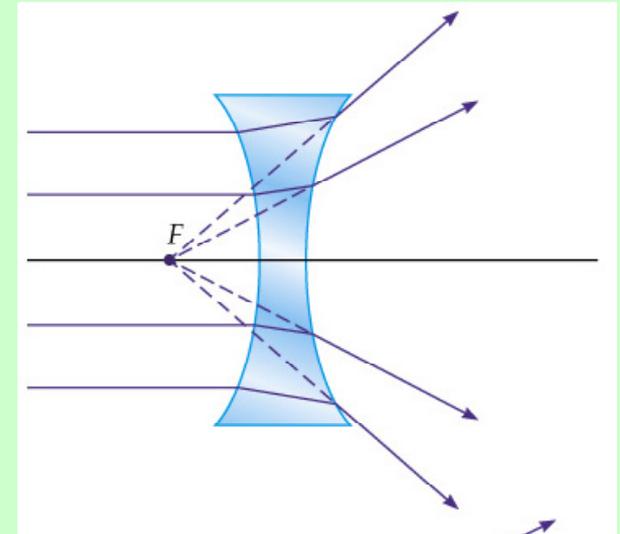


# Focal Point

The focal point of a lens is the place where parallel rays incident upon the lens converge.



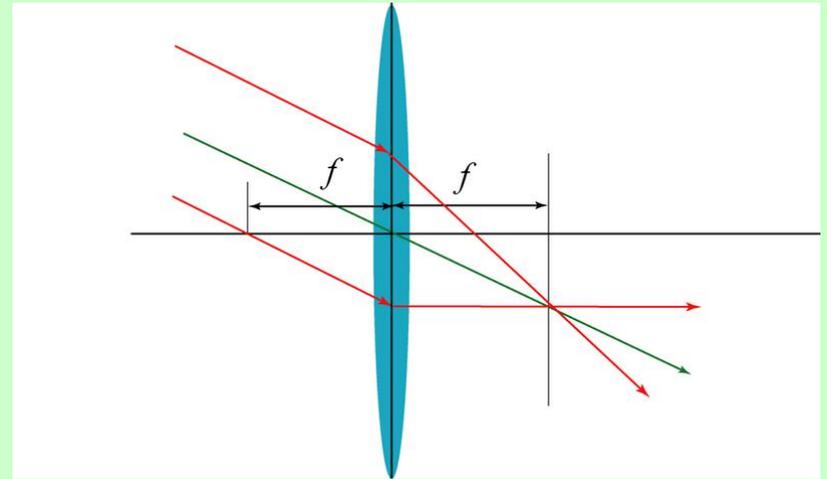
Converging or convex lens



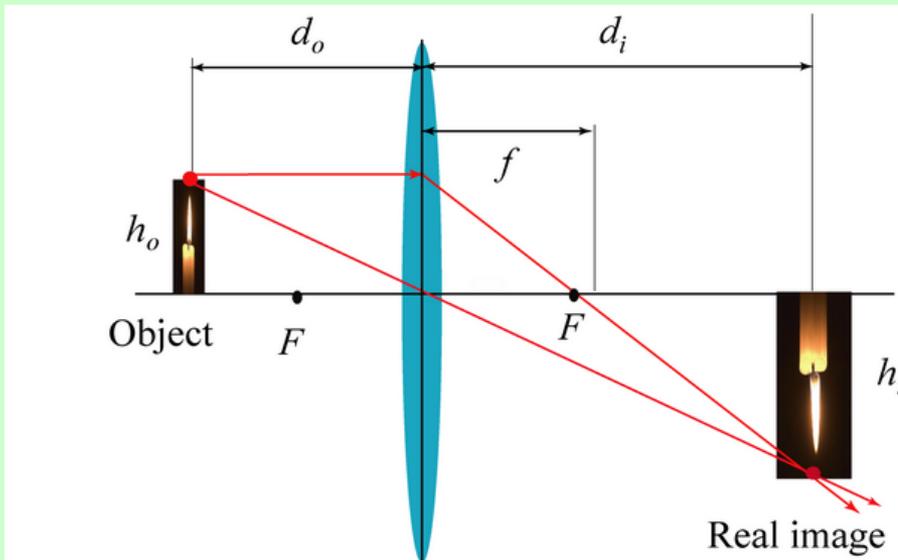
Diverging or concave lens

# Ray Tracing in a Thin Lens

1. Rays through lens center undeflected
2. Rays parallel to optic axis go through focal point
3. Parallel rays go to point on focal plane

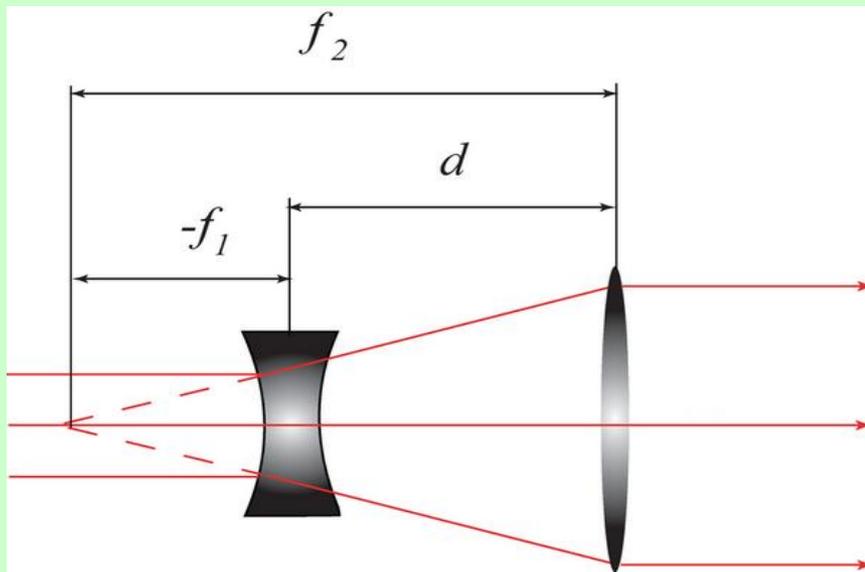


Focusing of the parallel inclined rays by thin lens

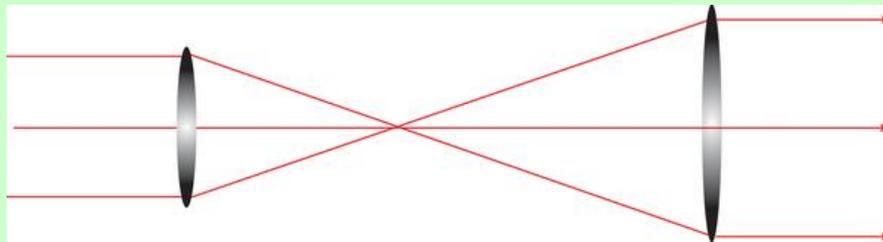


# Beam expanders

- Two types
    - (a) Galilaen and (b) spatial-filter arrangements
- Galilaen easier to set and maintain alignment

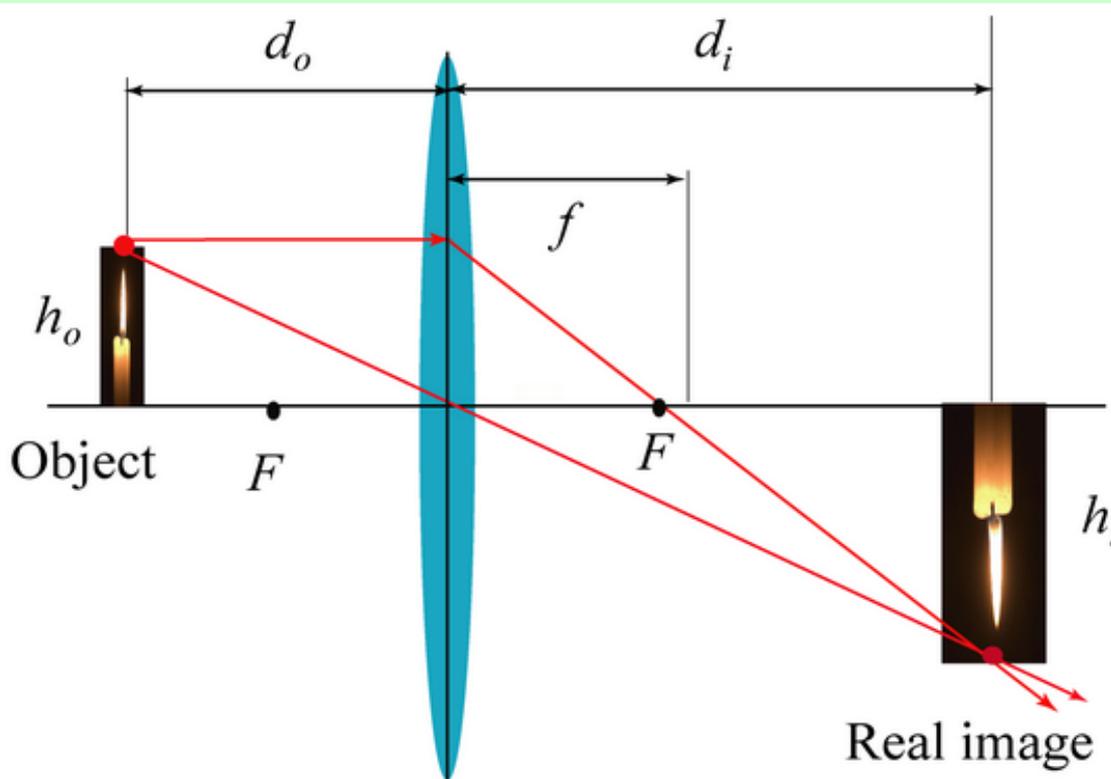


Galilaen arrangements



Spatial-filter arrangement

# Thin Lenses Magnification



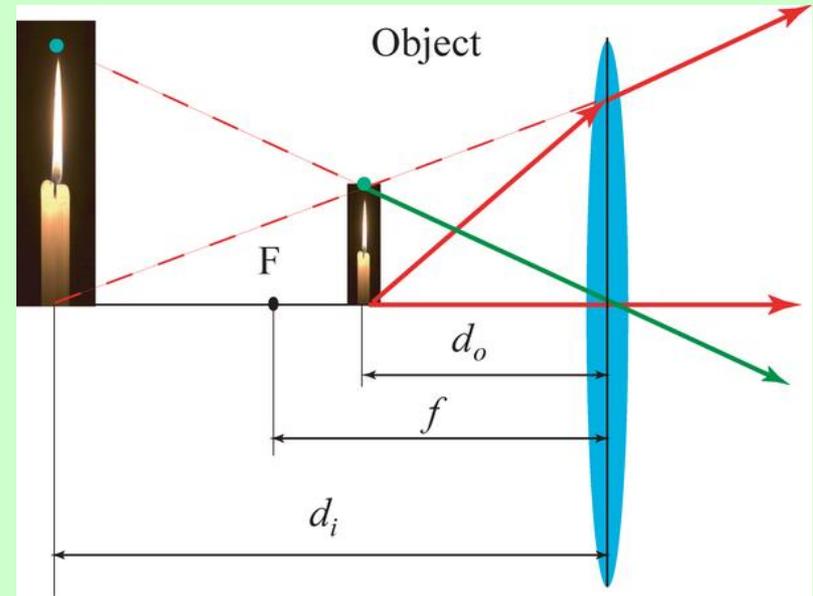
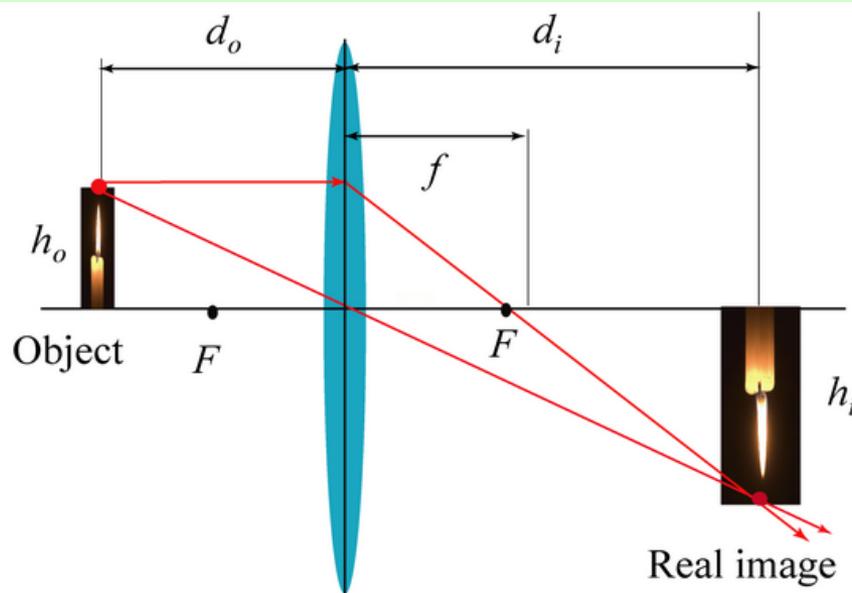
Thin lens equation

$$\frac{1}{d_o} + \frac{1}{d_i} = \frac{1}{f}$$

The linear magnification  $m$  is defined as the ratio of the image size  $h_i$  to the object size  $h_o$ :

$$m = \frac{h_i}{h_o} = -\frac{d_i}{d_o}$$

# Thin Lenses; Ray Tracing



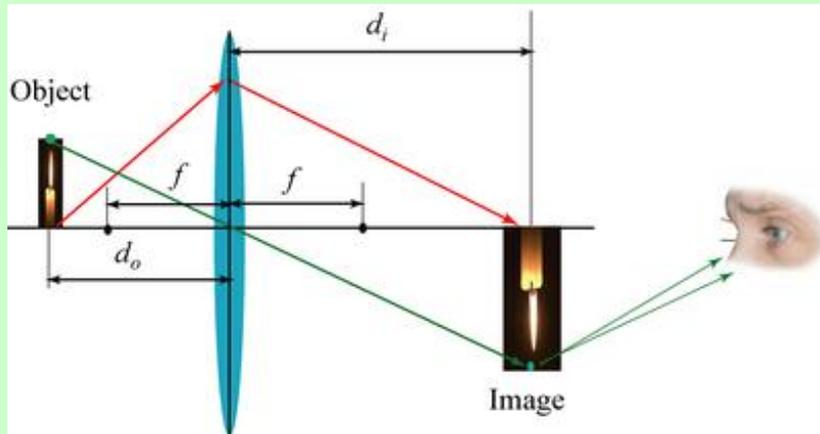
Ray diagram for locating the image of an object.

Left: the object is located outside the focal point of converging lens; the image is *real* and *inverted*.

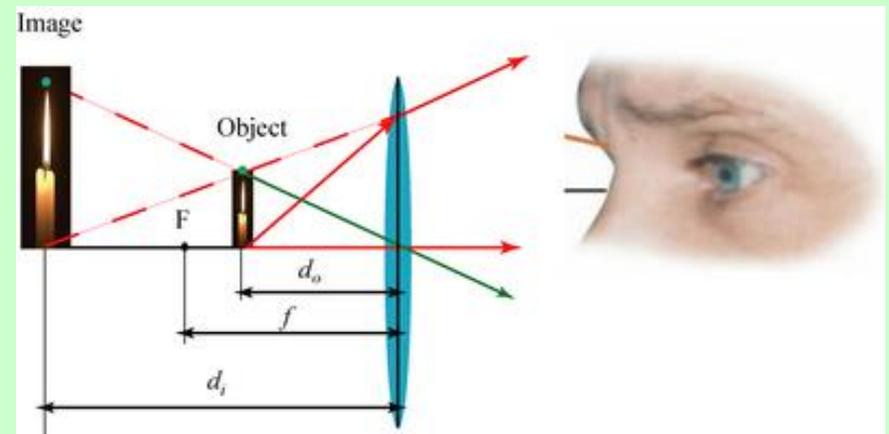
Right: the object is located inside the focal point of the converging lens; the image is *virtual*, *erect*, and *enlarged*.

# Real and Virtual Images

- Image properties to be concerned include
  - location, real/virtual, reduced/enlarged, upright/inverted, similar/distorted



**Real Image:** image lights actually pass through image



**Virtual Image:** image lights appear to have come from the image

➤ Real images can be formed on a screen.

# Magnification

If object is between the focal point and the lens., then the *magnification power* is

$$m = -\frac{d_i}{d_o}$$

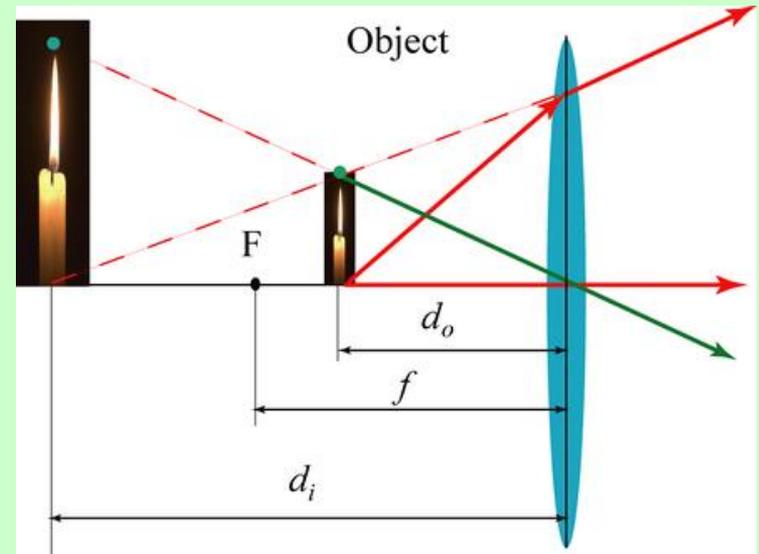
Image is virtual. Using thin lens formula

$$\frac{1}{d_o} + \frac{1}{d_i} = \frac{1}{f} \longrightarrow m = \frac{f}{f - d_o}$$

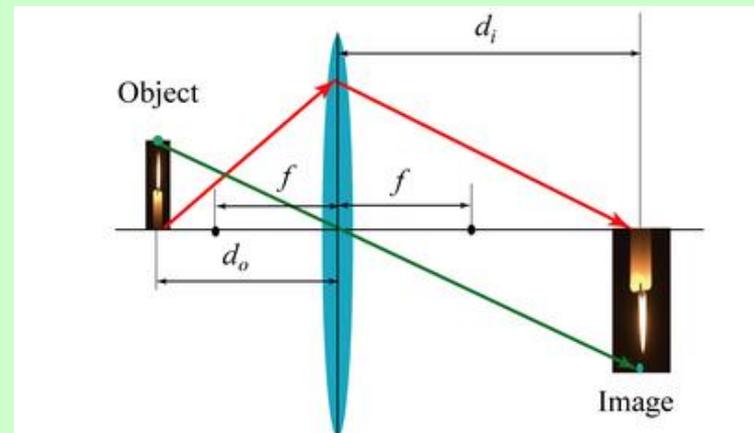
*If object is outside the focal point*

Image is inverted (negative) and real. Magnification is again

$$m = \frac{f}{f - d_o}$$



Object should be placed slightly inside the focal point.



# Magnification

Have you seen a 1000x magnifying glass  
What is wrong with it?

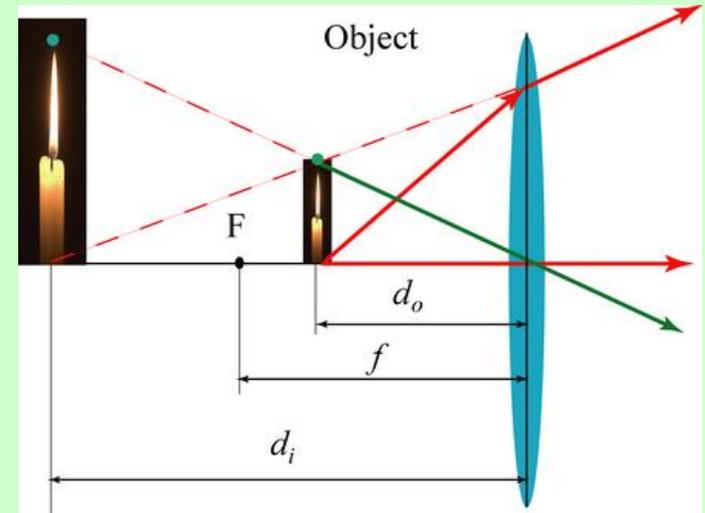
Try to make  $f - d_o$  small  $m = \frac{f}{f - d_o}$

$$d_o \rightarrow f \Rightarrow m = \frac{f}{f - d_o} \rightarrow \infty$$

Image is bigger, but it's far away. Doesn't help seeing more details. Usually  $d_i = 25$  cm for easy viewing

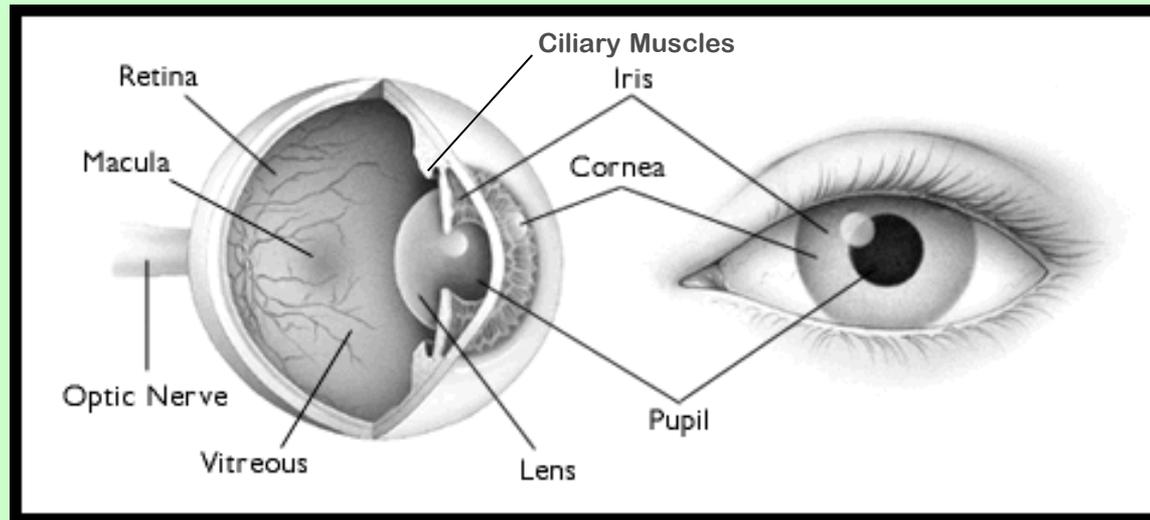
How about making  $f$  small?

Object must fit between  $f$  and the lens!

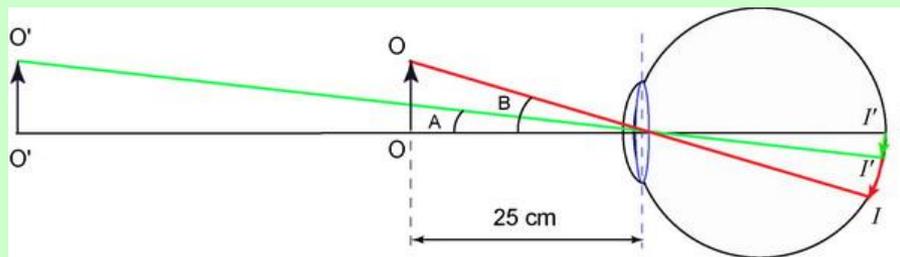


$$m = -\frac{d_i}{d_o} = \frac{25\text{cm}}{f}$$

# The human eye as an camera

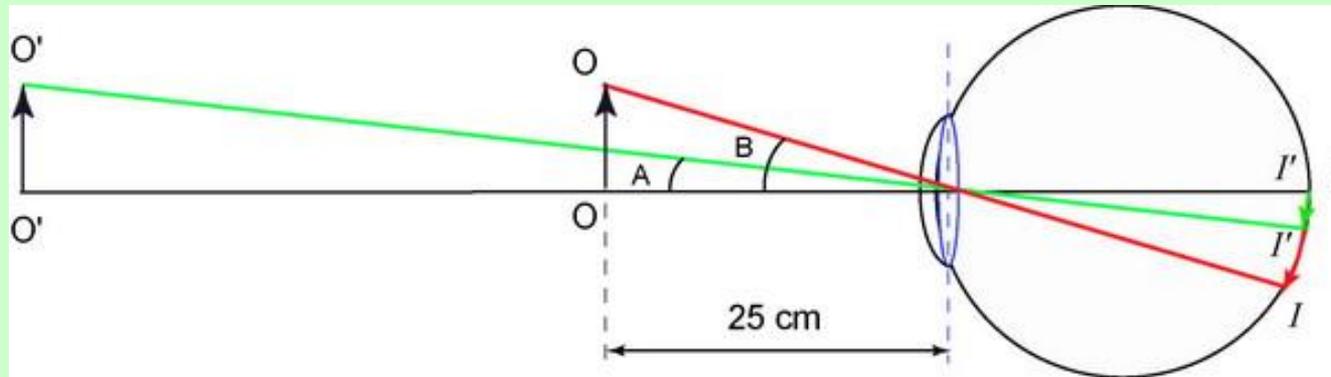


The human eye consists of a variable-geometry lens (crystalline) which produces a real image on a “screen” (retina) which is transmitted to the brain via the optical nerve. Iris - colored annulus with radial muscles. Pupil - the hole (aperture) whose size is controlled by the iris.



The crystalline automatically adjusts itself so we see well any object placed between infinity and a distance called “near point” (about 25cm for a typical 20 year old). The “image distance” is the eye diameter~2cm.

# The human eye as an camera



The visual angle subtended at the eye by two points  $O - O$  at *the nearest distance of distinct vision* (25 cm) is angle  $B$ ; if this exceeds about 1 minute of arc then the retinal image  $I - I$  will show the points as separate. If the same points are more distant ( $O' - O'$ ), then the visual angle  $A$  is less than one minute of arc and the points are not seen as separate.

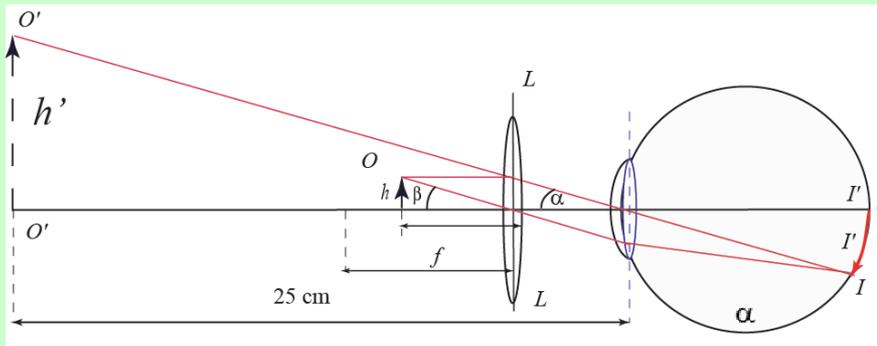
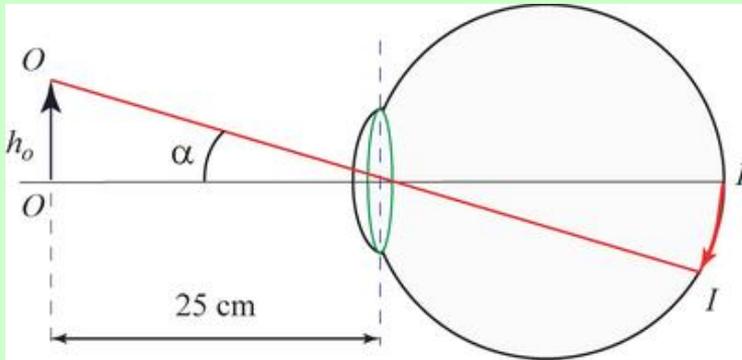
The maximum magnification of the eye:  $d_i$  (eye) = 2 cm, diameter,  $d_o = N = 25$  cm.

$$m = \frac{d_i}{d_o} = \frac{2}{25} = 0.08$$

# Увеличительное стекло

## Угловое увеличение

Если расстояние между объектом и линзой равно  $f$ , а изображение находится от глаза в точке наилучшего зрения,  $N = 25$  см, то расстояние объекта, соответствующее этому расстоянию изображения, может быть вычислено



$$\alpha = \operatorname{tg} \alpha = \frac{h'}{25 \text{ см}}$$

$$\beta = \operatorname{tg} \beta = \frac{h}{f}$$

$$m_{\theta} = \frac{\beta}{\alpha}$$

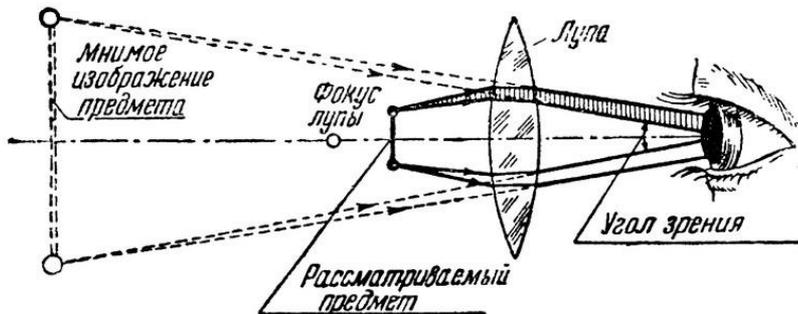


Рис. 12. Ход лучей в лупе.

$$m_{\theta} = \frac{25 \text{ см}}{f}$$

# Earliest Microscopes

•1673 - Antoni van Leeuwenhoek (1632-1723) Delft, Holland, is credited with bringing the microscope to the attention of biologists, even though simple magnifying lenses were already being produced in the 1500s. He worked as a draper (a fabric merchant. As a draper, he used a simple microscope to examine cloth. As a scientist, he began to experiment with new ways of grinding lenses in order to improve the optical quality. In total, he ground some 550 lenses, some of which had a linear magnifying power of 500 and a resolving power of one-millionth of an inch - an astounding achievement.

•The result of all this work was a simple, single lens, hand-held microscope. The specimen was mounted on the top of the pointer, above which lay a convex lens attached to a metal holder. The specimen was then viewed through a hole on the other side of the microscope and was focused using a screw.

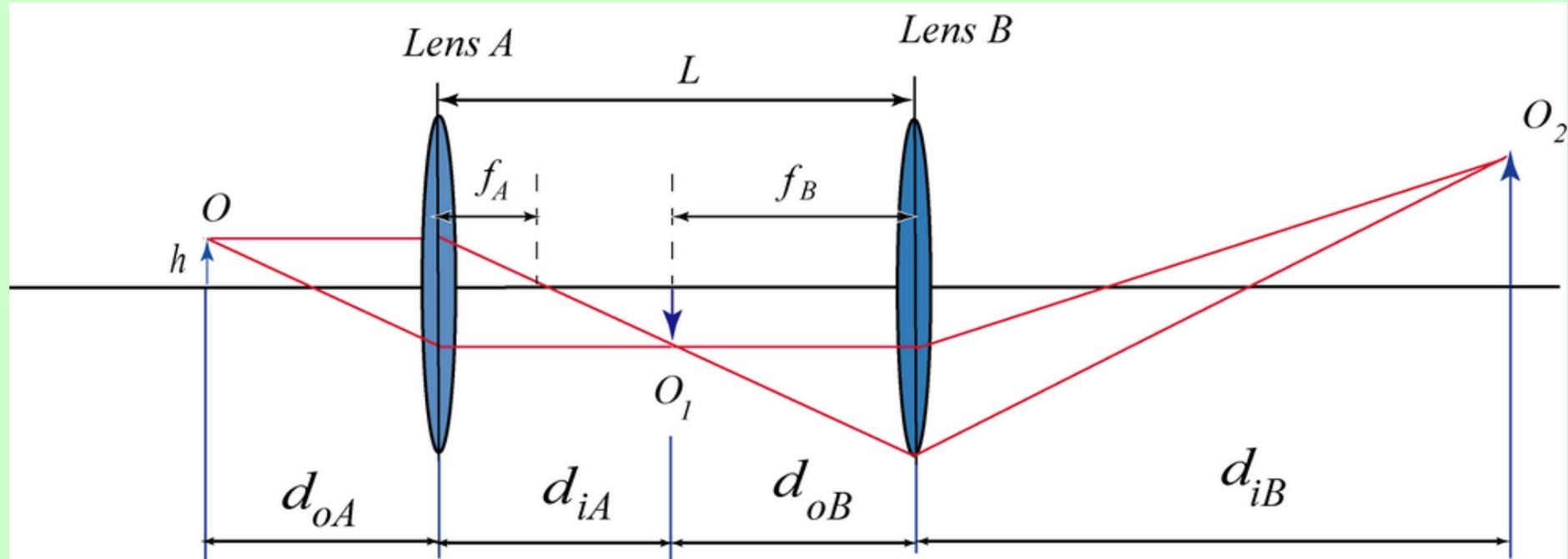
•It took until 1839, nearly two hundred years later, before cells were finally acknowledged as the basic units of life.



Leeuwenhoek Discovered bacteria, free-living and parasitic microscopic protists, sperm cells, blood cells, microscopic nematodes.

# Two Lenses and Compound Microscope

In lens combinations, the image formed by the first lens becomes the object for the second lens (this is where object distances may be negative).



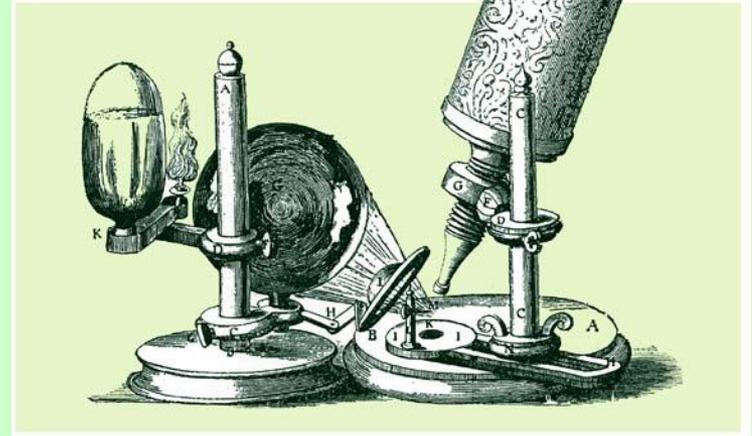
Linear *magnification* of two lenses

$$m = m_A \times m_B = \frac{d_{iA} \times d_{iB}}{d_{oA} \times d_{oB}}$$

# Compound Microscopes

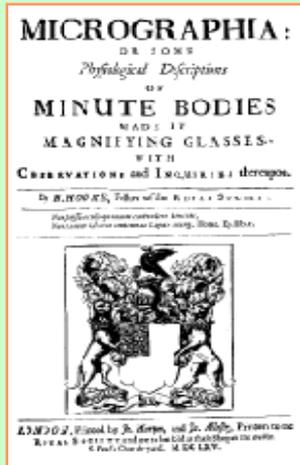


Robert Hooke's Microscope  
(1665)



1. ocular lens, or eyepiece
2. objective turret
3. objective lenses
4. coarse adjustment knob
5. fine adjustment knob
6. object holder or stage
7. mirror or light (illuminator)
8. diaphragm and condenser

# Earliest Microscopes



The earliest evidence of *magnifying glass* forming a magnified image dates back to the Book of Optics published by Ibn al-Haytham (Alhazen) in 1021. After the book was translated into Latin, Roger Bacon described the properties of magnifying glass in 13th-century England, followed by the development of eyeglasses in 13th-century Italy.

1590 - Dutch spectacle-makers Hans Janssen and his son Zacharias Janssen are often said to have invented the first *compound microscope* in 1590, but this was a declaration made by Zacharias Janssen himself during the mid 1600s. Another favorite for the title of 'inventor of the microscope' was Galileo

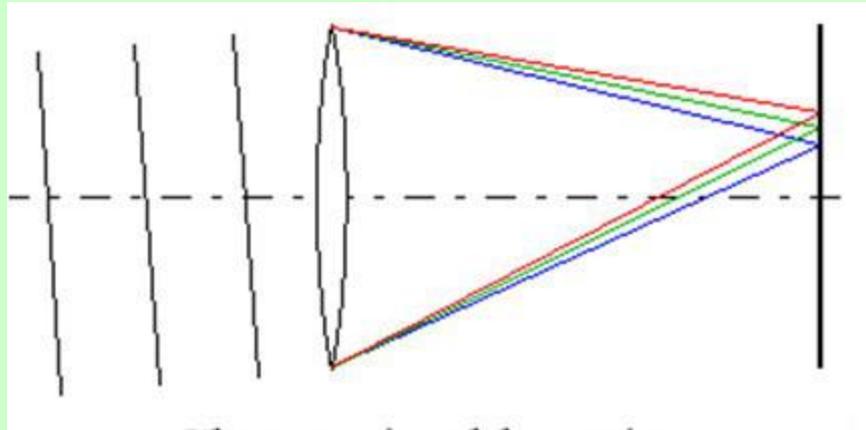
1660 - Marcello Malpighi (1628-1694), was one of the first great microscopists, considered the father embryology and early histology - observed capillaries in 1660 . Italian professor of medicine. Anatomist. First to observe bordered pits in wood sections. Gave first account of the development of the seed.

1665 - Robert Hooke (1635-1703)- book *Micrographia*, published in 1665, devised the compound microscope. Most famous microscopical observation was his study of thin slices of cork. Named the term “Cell”



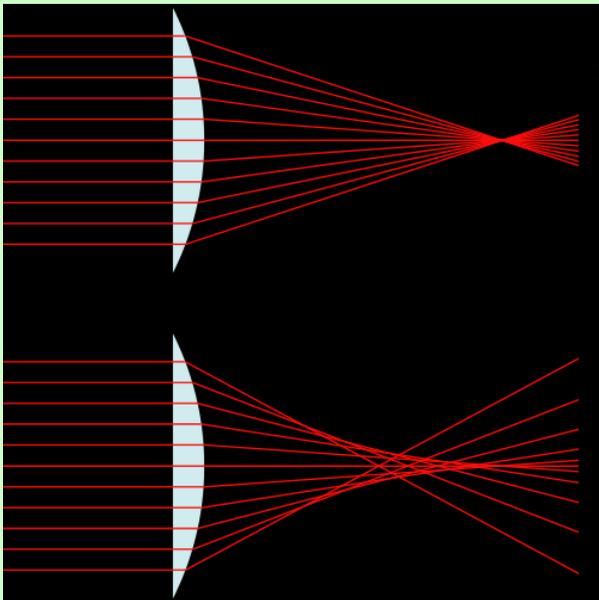
# The issues between simple and compound microscope

- **Simple microscopes** could attain around 2 micron resolution, while the best compound microscopes were limited to around 5 microns because of **chromatic aberration**
- In the 1730s a barrister names **Chester More Hall** observed that flint glass (newly made glass) dispersed colors much more than “crown glass” (older glass). He designed a system that used a concave lens next to a convex lens which could realign all the colors. This was the first ***achromatic lens***.



# The issues between simple and compound microscope

- Then in 1830, **Joseph Lister** solved the problem of spherical aberration (light bends at different angles depending on where it hits the lens) by placing lenses at precise distances from each other.
- Combined, these two discoveries contributed towards a marked improvement in the quality of image. Previously, due to the poor quality of glass and imperfect lens, microscopists had been viewing nothing but distorted images - somewhat like the first radios were extremely crackly.



Spherical aberration. A perfect lens (top) focuses all incoming rays to a point on the optical axis. A real lens with spherical surfaces (bottom) suffers from spherical aberration: it focuses rays more tightly if they enter it far from the optic axis than if they enter closer to the axis. It therefore does not produce a perfect focal point (Wikipedia, 2010).

# Ernst Leitz and Ernst Abbe

In 1849, **Karl Kellner** founded the Optical Institute in Wetzlar, Germany. Telescopes were the original emphasis, but within a few years microscopes took over as the main product. The company hired a very capable engineer named Ernst Leitz in 1865, who soon became a partner. Leitz took over the company in 1869 and renamed it Optical Institute of Ernst Leitz. By 1900, Ernst Leitz had produced 50,000 instruments.

**Carl Zeiss** (1816 – 1888) was an optician commonly known for the company he founded, *Carl Zeiss Jena*. Zeiss made contributions to lens manufacturing that have aided the modern production of lenses. In 1866 when Carl Zeiss recruited Ernst Abbe as his director of research at the Zeiss Optical Works. Abbe laid out the framework of what would become the modern computational optics development approach. He made clear the difference between magnification and resolution and criticized the practice of using eyepieces with too high a magnification as "empty magnification." By 1869, his work produced a new patented illumination device - the **Abbe condenser**.

Abbe's work on a wave theory of microscopic imaging (the Abbe Sine Condition) made possible the development of a new range of seventeen microscope objectives - three of these were the first immersion objectives and all were designed based on mathematical modeling.



# Further Development of Optical Microscopy

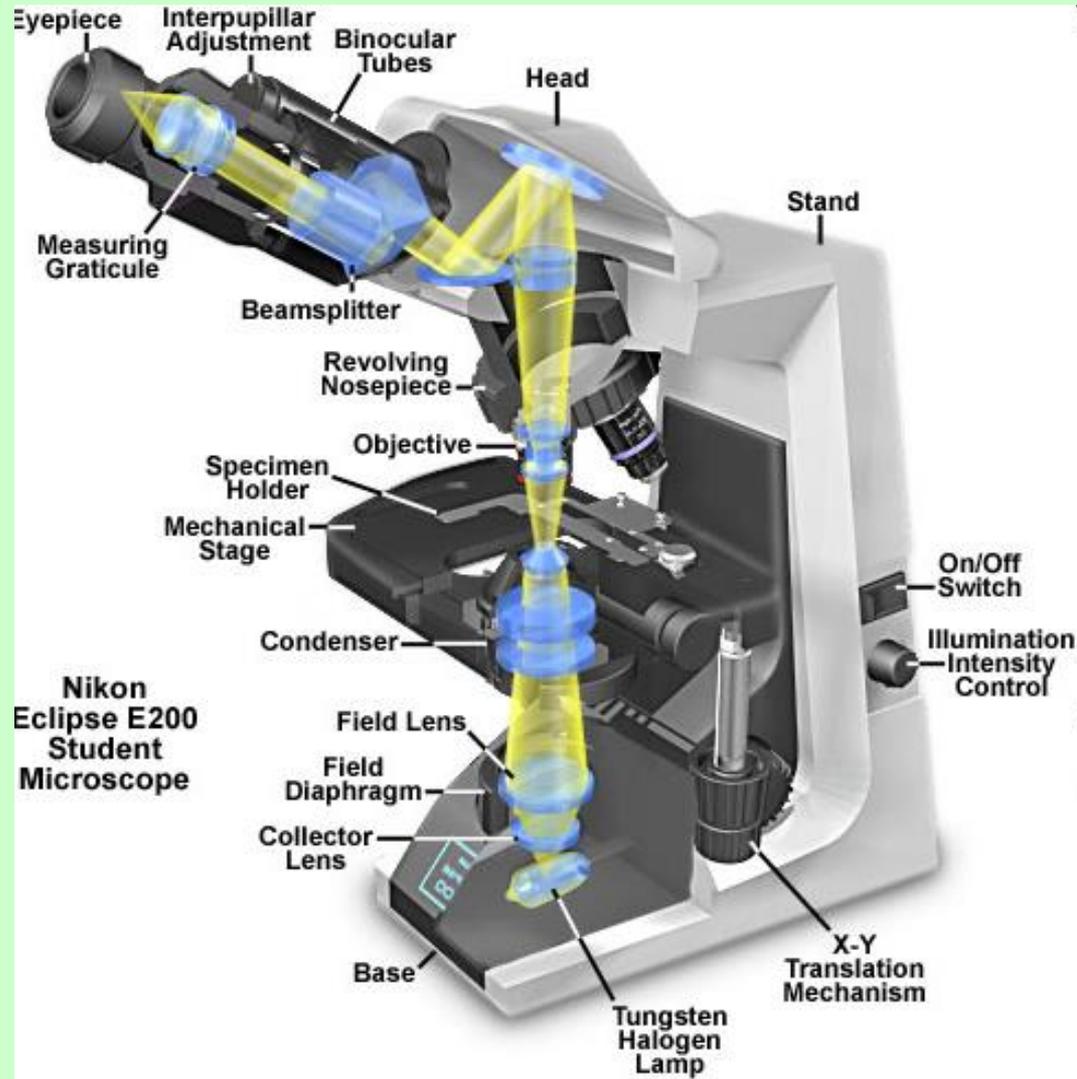
Abbe and Zeiss developed **oil immersion** systems by making oils that matched the refractive index of glass. Thus they were able to make the a Numeric Aperture (N.A.) to the maximum of 1.4 allowing light microscopes to resolve two points distanced only 0.2 microns apart (the theoretical maximum resolution of visible light microscopes). Leitz was also making microscope at this time.

**Kohler Illumination:** Devised by August Kohler in 1893 to use the full resolving power of the objective lens. It is method for properly aligning the light path such that the field is evenly illuminated and a bright image is obtained with and minimum glare and heating of the specimen



Zeiss student microscope 1880

# Further Development of Optical Microscopy



# Summary Lecture 1

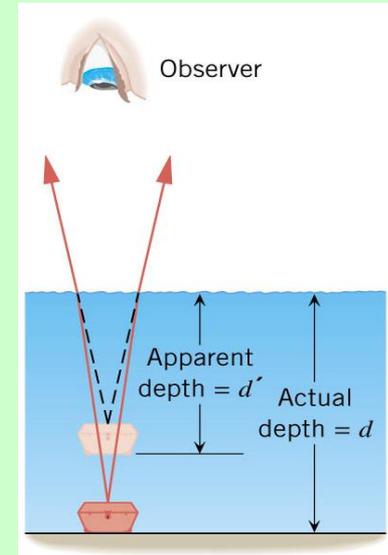
- Refraction, absorption, dispersion, diffraction
- Index of refraction
- Magnification of the magnifying glass
- Magnification of the compound microscope

## Reading:

1. R. A. Serway, J. S. Faughn. "*College Physics*". Saunders Cooleg Publ. (1985).
2. P. G. Hewitt. "*Conceptual Physics*". Pearson Prentice Hall (2005).
3. Olympus. Microscopy Research Center. [www.olympusmicro.com](http://www.olympusmicro.com)

# Home work

1. Introduce the concept of the *Total Internal Reflection* and describe practical application of it in optics.
2. Distance to the chest: How to determine the distance to the chest in water?
4. The beam of the green laser is directed toward diamond specimen. What is the wavelength of the laser beam inside diamond.
5. Find a position of the image of the objects that is located at the optical axes of a thin lens.



# Home work

6. Derive the thin lens formula using the sketch bellow

